IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s):	Guillermo J. Tearney et al.		
Application No.:	10/016,244		
Filing Date:	October 30, 2001	Examiner:	Shawna Jeannine Shaw
Title:	OPTICAL METHODS AND SYSTEMS FOR TISSUE ANALYSIS	Group Art Unit:	3737

DECLARATION UNDER 37 C.F.R. § 1.131

We, GUILLERMO J. TEARNEY and BRETT E. BOUMA, hereby declare as follows:

1. We are the joint inventors of the invention disclosed and claimed in U.S. Patent Application Serial No. 10/016,244 filed October 30, 2001 (the "'244 App."), which claims priority under 35 U.S.C. § 119(e) from U.S. Patent Application Serial No. 60/244,255 filed October 30, 2000 (the "'255 App.").

2. At the time the invention was made, we were employed by The General Hospital Corporation, the assignee of the entire right and interest to the above-identified application. We continue to be employed by The General Hospital Corporation.

3. We conceived the subject matter of the invention recited at least in independent claims 1 and 39 in the '244 App. (as amended in the attached Amendment) and described in the '255 App. on or before May 3, 2000. Further, the invention recited in pending claims 1 and 39 was reduced to practice at least as early as the filing date of the '255 application. We diligently worked on reducing the claimed invention to practice from the date of the conception thereof by providing the disclosure to a patent attorney, and working diligently with such patent attorney to file the '255 App.

4. In particular, on or before May 3, 2000, we conceived a method of analyzing tissue, in which a tissue is illuminated with coherent or partially coherent light, light reflected from the tissue is received at a detector, and a series of speckle patterns are formed, and changes in the speckle patterns are analyzed at time intervals sufficient to measure changes caused by microscopic motion of objects within the tissue, such that

the tissue is in vivo and/or the tissue is internal tissue (as recited in amended independent claims 1 and 39). This method was reduced to practice upon the filing of the '255 App.

5. In addition, on or before May 3, 2000, we conceived to practice a method of analyzing a tissue structure, in which a tissue is illuminated with coherent or partially coherent light, light reflected from the tissue is received at a detector, and a series of speckle patterns are formed such that the tissue is in vivo and/or the tissue is internal tissue, and speckle pattern data at time intervals sufficient to measure microscopic motion within the tissue structure or adjacent tissue, and the tissue structure is assessed by analyzing spatial characteristics of the speckle pattern data to deduce structural or biomechanical characteristics of the tissue structure (as recited in amended independent claim 39). This method was also reduced to practice upon the filing of the '255 App.

6. As evidence of the conception of the invention recited in amended independent claims 1 and 39 on or before May 3, 2000, attached hereto as Exhibit A is a copy of the eighteen (18) page presentation document entitled "Vulnerable Plaque Characterization Using Temporal and Spatial Speckle Analysis" (referred to herein below as "Presentation") which was prepared internally at least as early as May 3, 2000.

7. The attached document demonstrates that we conceived the method of the invention to analyze tissue and tissue structure at least as early as the completion date of the Presentation (i.e., on or before May 3, 2000). For example, the Presentation describes a receipt of a coherent interference of light remitted from a scattering media or substrate. (see, e.g., Presentation, p. 7). Further, the Presentation describes the formation of speckle patterns to be detected by indicating that, e.g.,

> "Motion of a single scatterer in the specimen changes the speckle pattern
> • The time dependent speckle pattern can be used to determine the Brownian motion within a multiply scattering media
> • The motion is characterized by the spatial decorrelation of the speckle pattern as a function of time
> • For Brownian motion, the decorrelation is a negative exponential with a time constant, τ" (see *id.*)

8. In addition, the Presentation describes a determination of microscopic motion within the tissue and/or adjacent to the tissue, and also determining the spatial characteristics of speckle pattern data. For example, it is provided as follows:

"Spatial and Temporal Characterization of Plaques

Measuring the speckle decorrelation time, τ, as a function of distance from beam entry point allows measurement of Brownian Motion and
- Cap thickness
- Cap stiffness
- Lipid pool stiffness" (see *id.*, p. 9).

9. The tissues being discussed in the Presentation are clearly provided in vivo and/or are internal tissues by referring to IVUS and OCT techniques which measure the tissues in vivo and/or internal tissues. Further, the Presentation describes the determination of the structural or biomechanical characteristics of the tissue structure. (See *id.*, pp. 3-5).

10. The invention as recited in now-pending independent claims 1 and 39 has been reduced to practice by filing the '255 App., which completely describes each of the features recited in these claims. For example, the '255 App. (a copy of which is attached herewith as Exhibit B), at least on pages 1-4 as well as in other portions thereof, clearly describes each and every feature recited in these claims.

11. We further declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements are made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing thereon.

6/3/05
Date

GUILLERMO J. TEARNEY

6/3/05
Date

BRETT E. BOUMA

Vulnerable Plaque Characterization Using Temporal and Spatial Speckle Analysis

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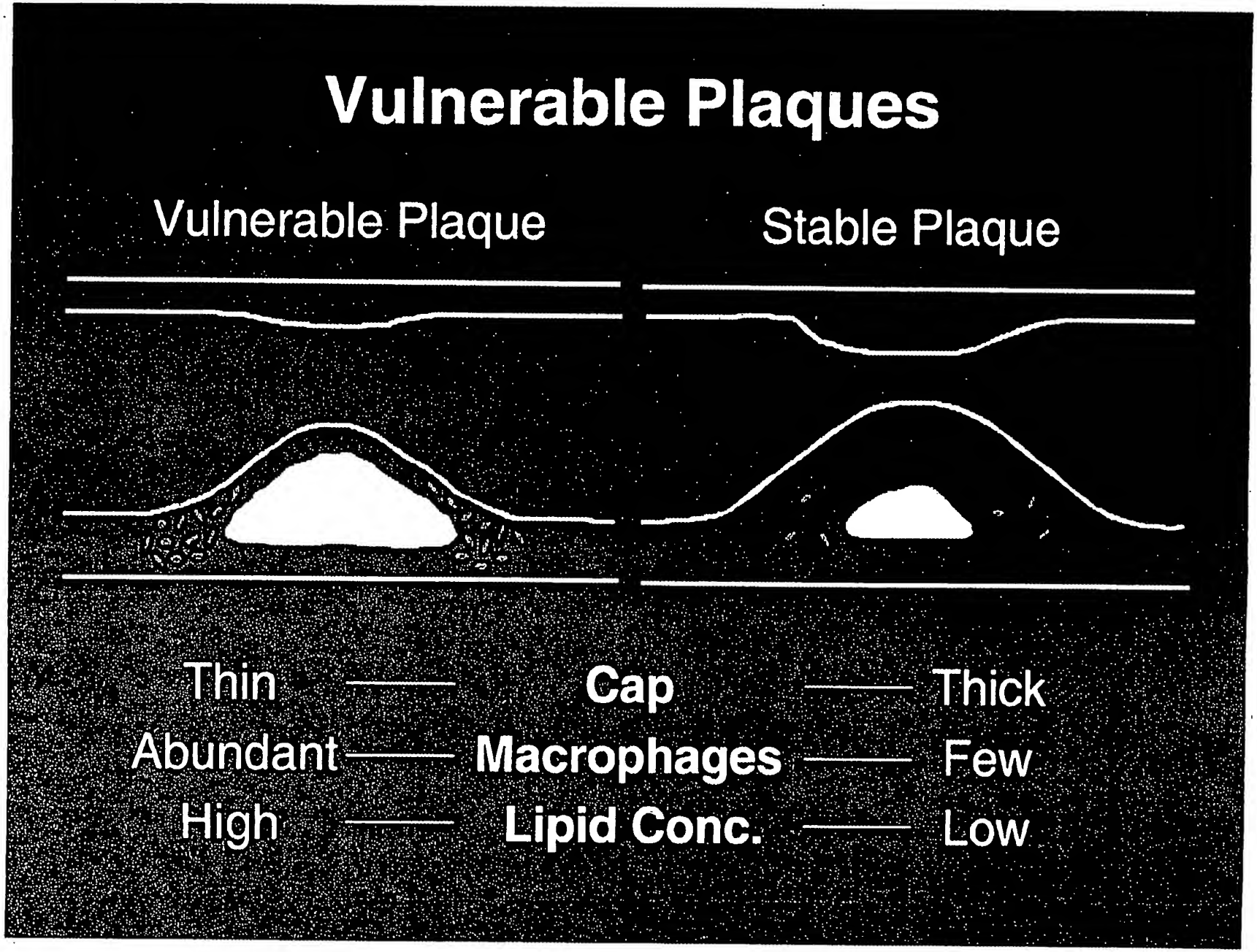
Department of Pathology and
Wellman Laboratories of Photomedicine

Vulnerable Plaques



Vulnerable Plaque Diagnosis

Proposed Diagnostics

- Infrared
  - Indirectly measures lipid content of plaque
- Fluorescence
  - Measures autofluorescence
    - Collagen
    - MMP
- IVUS
  - Structural measurement of cap
  - Poor resolution
- OCT
  - Structural measurement of cap
  - Sufficient resolution for measurement of cap thickness

Proposed methods do not measure the biomechanical properties of plaque

Intrinsic Plaque Biomechanics

Biomechanical properties

- Cap strength
  - Proportional to thickness and structural integrity
- Lipid pool
  - Shear stress and strain on cap are related to lipid pool stiffness
  - Rupture of plaque tends to occur in areas of large stiffness gradient between cap and lipid pool
  - Lipid lowering drugs increase stiffness of lipid pool
  - Stiffening of the lipid pool decreases vulnerability

Mechanical stiffness of the cap and lipid pool are essential parameters for assessing the likelihood of plaque rupture

Viscosity

Viscosity of tissue is proportional to stiffness

- Related to the ability of the molecules in the tissue matrix to move

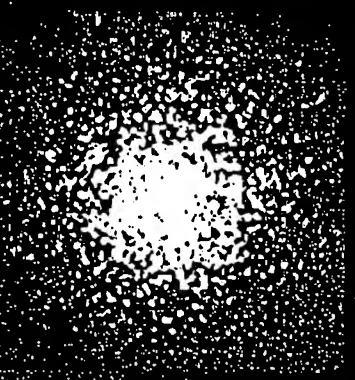
Brownian motion

- Random motion of particles in the matrix
- Brownian motion is inversely proportional to viscosity and stiffness
  - **Low stiffness, rapid Brownian motion**
  - **High stiffness, slow Brownian motion**

Brownian motion velocity is a measurement of tissue stiffness

Speckle

Coherent interference of light remitted from a scattering media or substrate



- Produces a grainy pattern at the surface of the specimen and in the image plane
- The pattern is created from the remitted field after many multiple scattering events within the specimen
- Motion of a single scatterer in the specimen changes the speckle pattern

Speckle Motion

Motion of a single scatterer in the specimen changes the speckle pattern

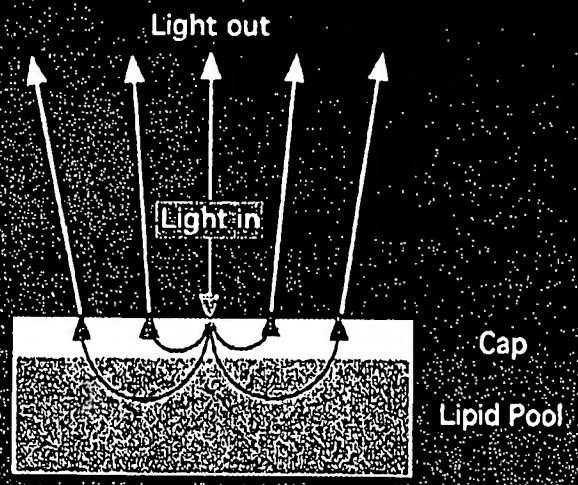
- The time dependent speckle pattern can be used to determine the Brownian motion within a multiply scattering media
- The motion is characterized by the spatial decorrelation of the speckle pattern as a function of time
- For Brownian motion, the decorrelation is a negative exponential with a time constant, τ

Stiffness of the cap and lipid pool can be determined by measuring the speckle decorrelation time constant

Light Diffusion

In tissue, light remitted further from the beam entry point has probed deeper into the tissue

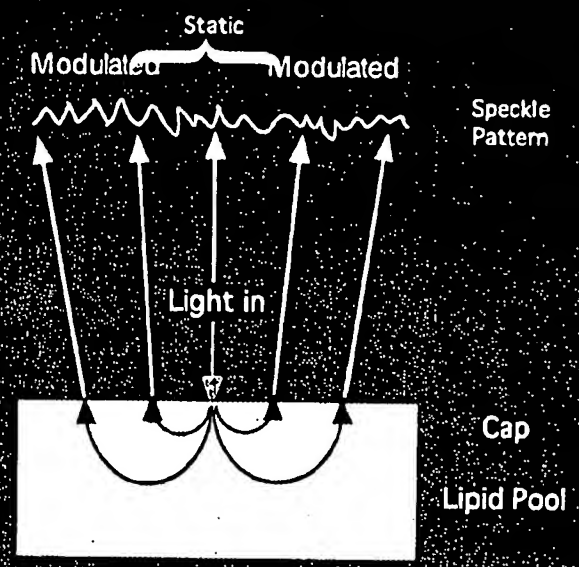
- Governed by the optical properties of tissue



Spatial and Temporal Characterization of Plaques

Measuring the speckle decorrelation time, τ, as a function of distance from beam entry point allows measurement of Brownian Motion and

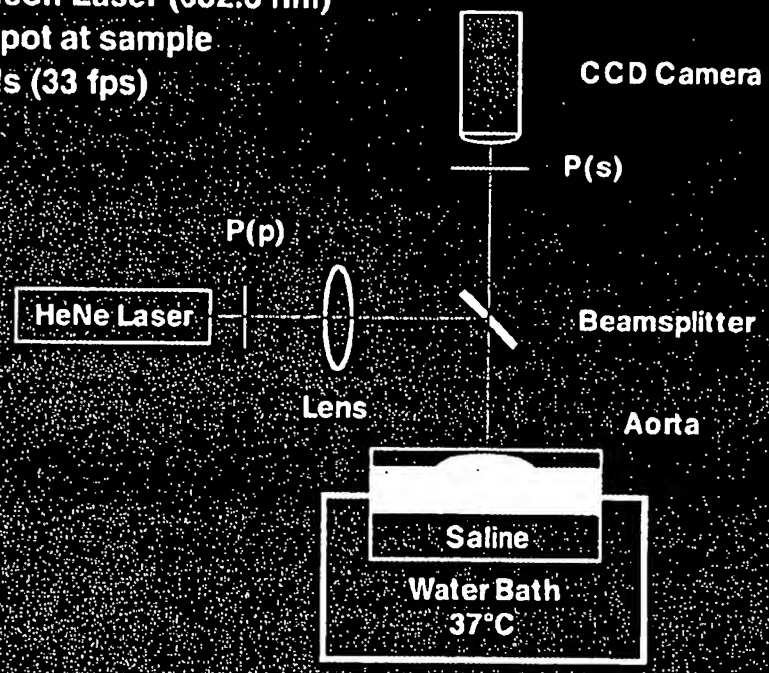
- Cap thickness
- Cap stiffness
- Lipid pool stiffness



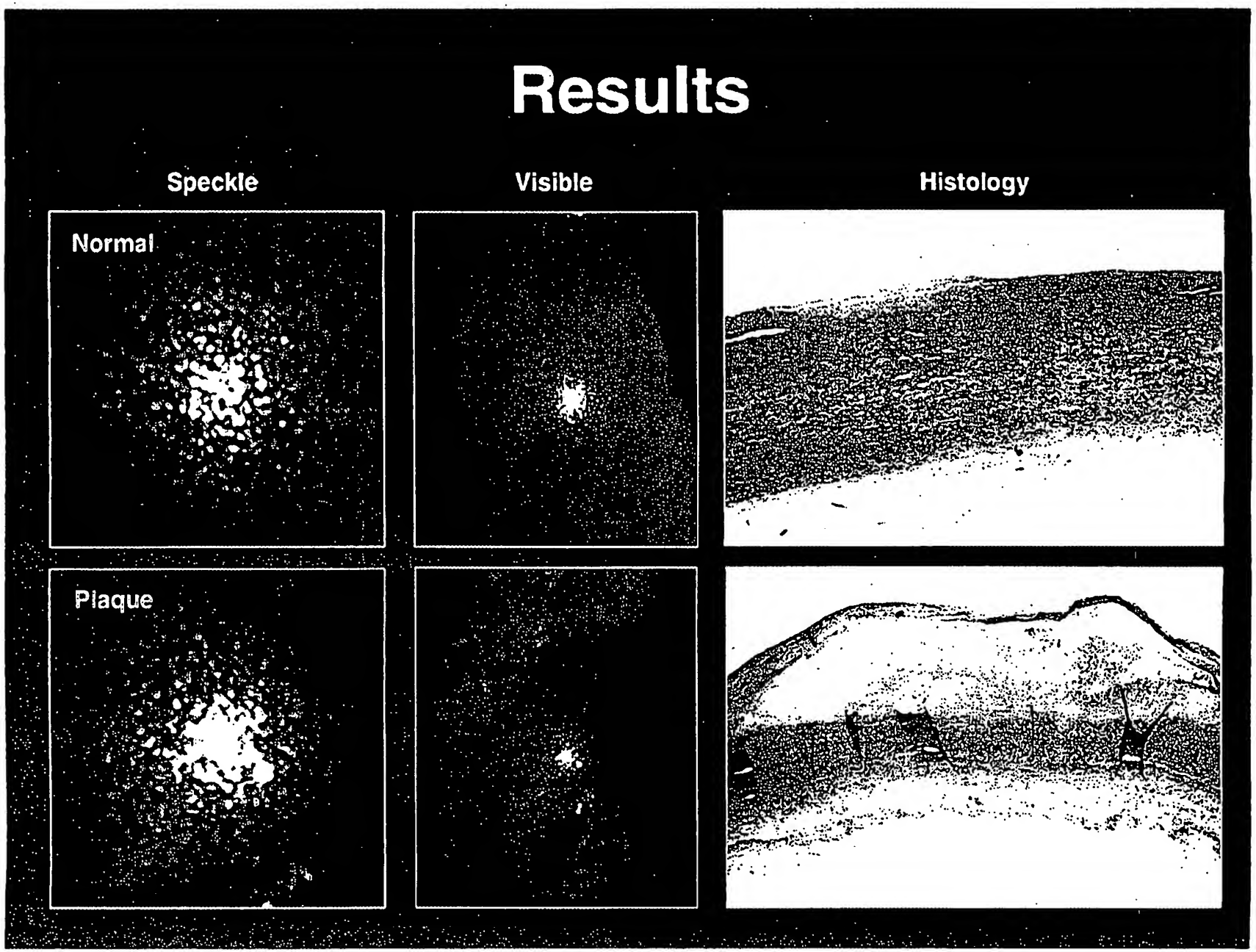
Proof of Principle

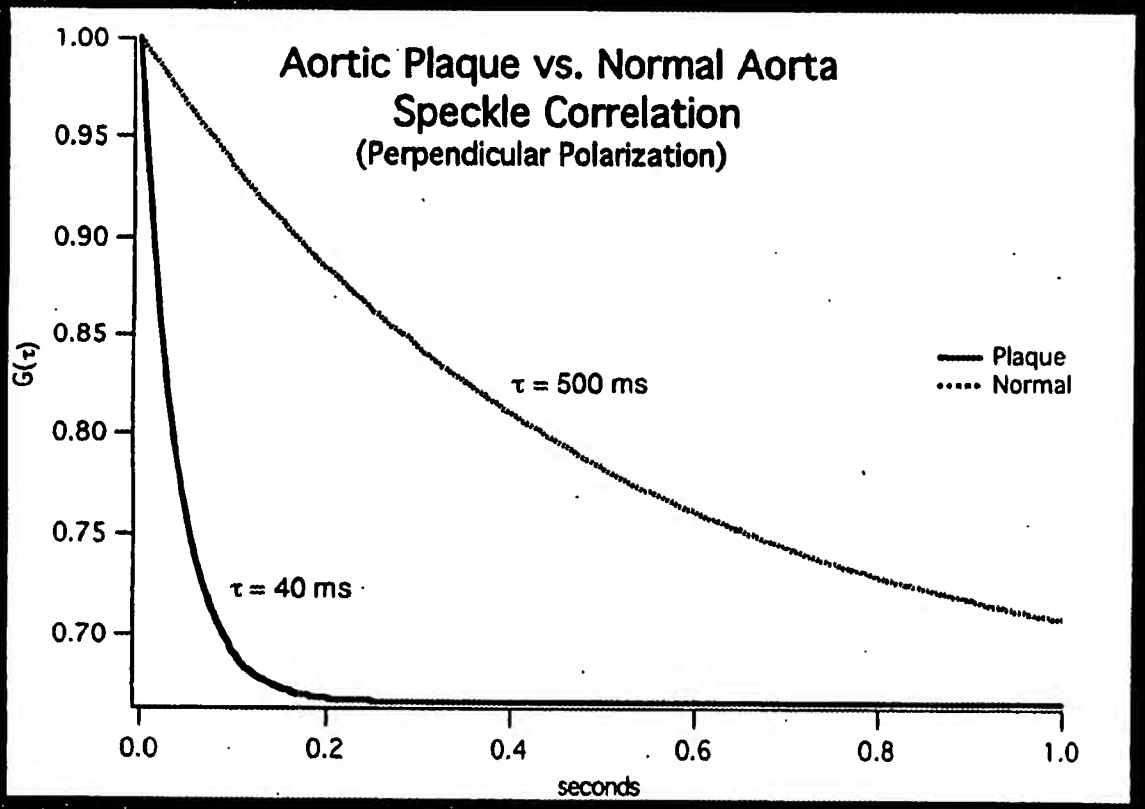
Methods

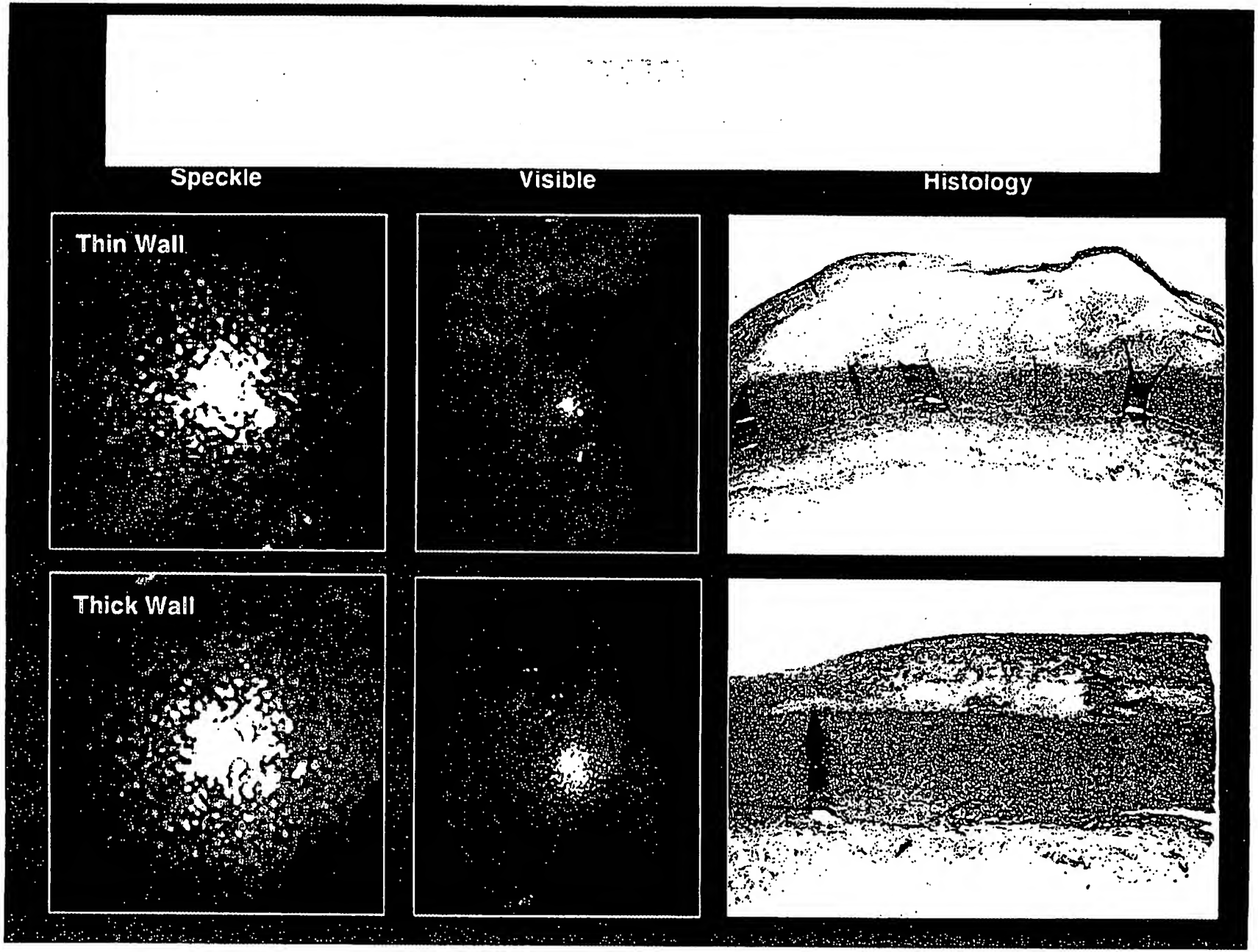
- Cadaveric aortas
- Normal saline, 37°C
- Helium Neon Laser (632.8 nm)
- 100 μm spot at sample
- 2 seconds (33 fps)

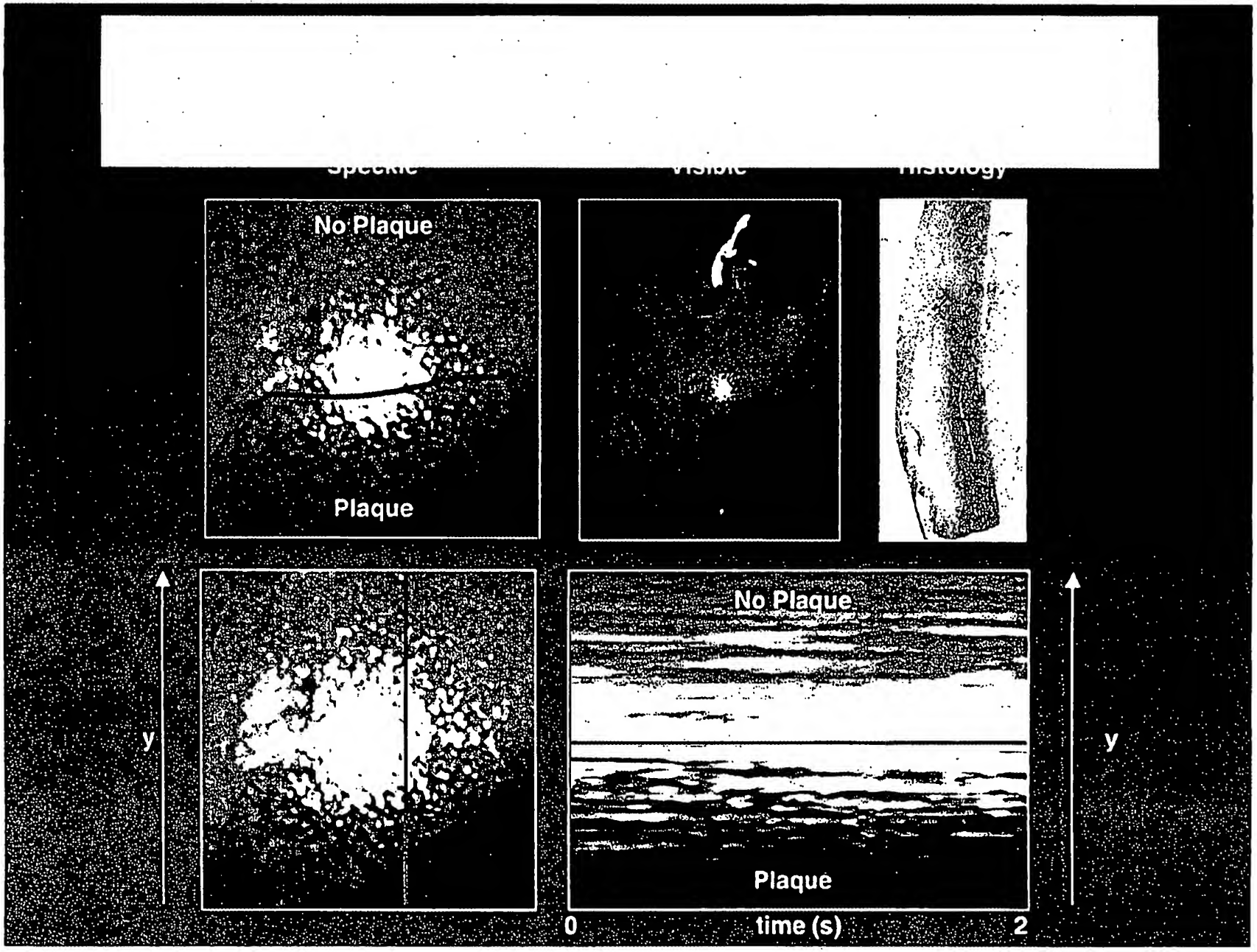


Results




Aortic Plaque vs. Normal Aorta
Speckle Correlation
(Perpendicular Polarization)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
G(τ)
τ = 500 ms
τ = 40 ms
Plaque
Normal
0.0
0.2
0.4
0.6
0.8
1.0
seconds


Speckle
Visible
Histology
Thin Wall
Thick Wall


No Plaque
Plaque
y
No Plaque
Plaque
y
0
time (s)
2

Feasibility Study Summary

Speckle decorrelation time constant is different between normal aorta and plaque

- τ = 500 ms vs 40 ms

Speckle decorrelation time constant is different between thin and thick-walled plaques

- Greater for thick-walled plaques

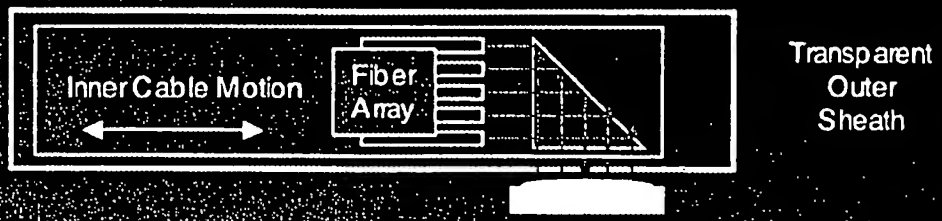
Speckle decorrelation is spatially dependent

- Border between plaque and normal aorta demarcates different speckle decorrelation time constants

Clinical Realization

Catheter based technique (one possibility)

- Array of fibers
- Scanned probe



Difficulties

- Intrinsic heart and catheter motion
  - **Lipid pool Brownian motion time constant is approximately 40 ms**
- Blood
  - **Will need saline infusion and/or direct contact with tissue**

Localize time and space (x, y, z) dependent speckle patterns using optical methods as opposed to light diffusion

- Confocal microscopy
  - Apertures in the source and detector planes combined with a high numerical aperture imaging lens
  - High resolution speckle analysis in (x, y, z)
  - Speckle decorrelation is less sensitive than multiple scattering technique
- Optical Coherence Tomography (OCT)
  - Uses low coherence interferometry to obtain localization in z
  - Measures cap thickness directly
  - Speckle decorrelation is less sensitive than multiple scattering technique

Conclusion

Temporal and spatial analysis of the speckle patterns can potentially determine

- Cap thickness
- Cap and plaque viscosity
- Spatially resolved biomechanical stiffness
- Plaque vulnerability

Future work

- Speckle statistics
  - **Can determine cap thickness and optical properties**
  - **Low coherence light**
- Strain and stress measurements
  - **Correlate biomechanical properties with Brownian motion measured by speckle decorrelation**
- Probe development
- Continue cadaveric aorta studies
- In vivo studies (e.g. rabbit model)